Supplemental Appendix

Correlation between qPCR and flow cytometry

Across all 3 studies, a correlation was observed between the transgene level from qPCR and the cell surface expression of CAR from flow cytometry in PB summarized both by individual patient AUC0-28d (r = .7222; P < .0001) and Cmax (r = .6371; P < .0001) values (Supplemental Figures 1A and 1B). This indicates an overall concordance between these 2 methods; however, there are some exceptions in which there is expansion observed by qPCR but little to no expansion measured by flow cytometry (Supplemental Figure 3, right panel). This difference may arise because these 2 techniques measure 2 different analytes (transgene level by qPCR and surface expression of CAR by flow cytometry) and possess different validation parameter ranges, including the limits of quantification.

Cellular kinetics by event-free survival in pediatric B-ALL

Cellular kinetic profiles by event free survival (EFS) categories (Supplemental Figure 2) suggests that patients with longer CTL019 persistence maintained longer EFS. In EFS analysis, patients with a relapse occurring < 6 months after CTL019 infusion had lower overall persistence than patients with an EFS of > 6 months. At month 6, there is a separation in the curves for patients with an EFS of < 6 months (patients with relapsed disease before 6 months) and patients without events after 6 months.

Cellular kinetics by response in patients with CLL

In patients with CLL, median T1/2 and Tlast of the CTL019 transgene were longer for patients with CR/CRi vs PR/PRi vs NR/PD (Table 1); however, median Tmax was similar across response groups. Higher exposure to circulating CTL019 T cells was observed for responding patients (CR/CRi and PR/PRi)

compared with nonresponding patients (NR/PD). Although the sample size of the NR/PD group was small, there was a clear trend for higher expansion in patients with CR/CRi relative to NR/PD, by both transgene levels and flow cytometry (Figure 2). For example, the geometric mean (CV%) Cmax was higher in patients with CR/CRi (27,400 [478] copies/µg DNA) and PR (37,300 [260] copies/µg DNA) compared with NR/PD (578 [744] copies/µg) DNA. The corresponding flow cytometry geometric means (CV%) for C_{max} in CR/CRi, PR/PRi, and NR/PD patients were 9.2% (262), 10.6% (2210), and 0.5% (282), respectively, for the $CD3^+$ $CTL019^+$ T cells. Similarly, means for AUC0-28d and AUC0-84d were also higher in patients with CLL with CR/CRi and PR compared with NR/PD patients. Median (CV%) AUC0-28d was 168,000 (560) copies/µg DNA × days for patients with CR/CRi, 425,000 (395) copies/µg DNA × days for patients with PR/PRi, and 5010 (1110) copies/µg DNA × days for patients with NR/PD.

Cellular kinetics by age categories

The impact of age on the cellular kinetics of CTL019 was evaluated using the following age categories: <6 years, ≤6 years, ≥6 to <13 years, ≥13 to <18 years, ≥18 years of age. Similar AUC0-28d and Cmax were observed for patients across age categories, suggesting that age does not impact CTL019 expansion (Supplemental Table 5).

Influence of extrinsic factors on expansion

The impact of several extrinsic factors (prior stem-cell transplant (SCT), presence/absence of lymphodepleting chemotherapy) was assessed. Overall, there was no difference in expansion between patients with prior SCT (n = 36) and those without prior SCT (n = 18). Additionally, similar expansion was seen in patients with fludarabine based LD chemo (n = 42) compared with non-fludarabine based LD (n = 8) (Supplemental Figure 8).

Cellular kinetics by number of CTL019 doses infused within 28 days

Patients enrolled in this trial received fractionated doses of CTL019 per the protocol. Fractionated doses were administered based on the individual patient's tolerability. The cellular kinetics (Cmax, AUC0-28d and Tmax) were categorized by the number of CTL019 doses patients received within the first 28 days. These results show consistent expansion across the dose categories. Similarly, the Tmax occurred at around day 10-11 (Supplemental Table 6), consistent with prior observations.[1]

T-cell viability in final product

T cell viability was assessed as part of the release criteria for CTL019. The range of T cell viability was 73.2% to 95.8% with a median of 87.1%. Following infusion, patients (ALL and CLL) experienced a multi log expansion of CAR T cells; product T cell viability did not appear to be a factor impacting in vivo expansion. (Supplemental Figure 9).

Impact of tocilizumab on cellular kinetics

Fifteen patients with pediatric B-ALL (CR/Cri) received tocilizumab for the management of CRS. In vivo expansion and persistence of CTL019 T cells were not diminished in CR/CRi patients treated with tocilizumab compared with CR/CRi patients who did not receive tocilizumab, as measured by qPCR. Responding patients treated with tocilizumab had approximately 3.8- and 2.5-fold higher AUC0-28d and Cmax, respectively, compared with those not treated with tocilizumab (Table 3). Tocilizumab is administered to patients with grade 3/4 CRS, and these patients tend to have greater expansion, higher Cmax and AUC0-28d, and often higher preinfusion tumor burden. There is limited experience with tocilizumab administration in adult ALL to make definitive conclusions on its impact on cellular kinetics—

only 1 adult patient with ALL received a single dose and 2 patients received 2 doses of tocilizumab. Six patients with CLL received tocilizumab and had a geometric mean (CV%) Cmax of approximately 50,095 copies/µg and AUC0-28d of 572,657 copies/µg × day. Compared with the overall population of patients presented in Table 1, patients treated with tocilizumab had higher exposure compared with all response categories. In addition, corticosteroids were administered to patients who did not fully respond to a first dose of tocilizumab, per the CRS treatment algorithm. Corticosteroids were administered at low doses (1-2 mg/kg/day) over a short duration and were weaned rapidly over several days. Patients who received tocilizumab and corticosteroids continued to show CTL019 expansion and persistence (Table 3).

Impact of conditioning regiments on cell kinetics

Of the 3 protocols, only one required a single predetermined LD conditioning chemotherapy (adult ALL) while the pediatric ALL and CLL protocols allowed physicians choice of LD regimen. In adult ALL, 5 patients received cyclophosphamide and 1 patient received clofarabine. In CLL, conditioning regimens were typically fludarabine/cyclophosphamide or bendamustine. Additionally, 3 patients with CLL received ibrutinib for 5 to 15 months prior to CTL019 infusion. CLL had progressed in 2 of these patients and was stable in one patient treated with ibrutinib prior to CTL019 infusion. Two patients stopped ibrutinib within one week of the collection of T cells (apheresis); one patient responded (PRi) to CTL019 and one did not. One patient stopped ibrutinib 2 months prior to T cell collection due to progression on ibrutinib and subsequently had a PR following CTL019 infusion. It was not possible to robustly assess the impact of LD conditioning therapy type or prior ibrutinib therapy on cellular kinetics in those studies due to small patient numbers and subgroups. In the pediatric ALL trial, the majority of patients received fludarabine with cyclophosphamide (FC) (n = 42, 78.7%) while 8 patients (14.2%) received a non-fludarabine based regimen. In addition, a small subset (4 patients, 7.1%) of patients did not receive LD

conditioning chemotherapy since lymphopenia was already present just prior to CTL019 infusion. Supplemental Figure S8 panels C and D show a comparison of the exposure of transgene across conditioning regimens in pediatric ALL. There were minimal differences in exposure by conditioning regimen. However, it is important to note that this subgroup of non-FC or no LD chemotherapy patients is limited in number.

Supplemental Discussion

Assessment of the relationship between infused CAR T cell dose and in vivo expansion with clinical toxicity and efficacy are important to consider in the development of T-cell directed therapies and in the registration of such products. These assessments, however, have some unique features. For example, there is very little relationship between dose of CTL019 and efficacy in CLL.[2] Additionally, we previously showed that there is no relationship between CTL019 dose and expansion for pediatric B-ALL.[1] CAR T cells as "living drugs" expand beyond the initial dose infused (up to 10-fold or more) and therefore in vivo CART T cell levels, function and persistence are more likely to be important than the infused dose.

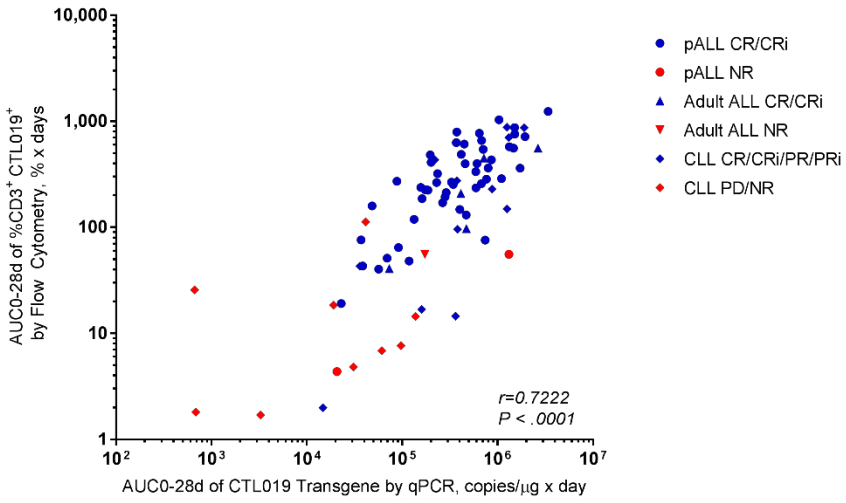
References

1. Mueller KT, Waldron E, Grupp SA, et al. CTL019 clinical pharmacology and biopharmaceutics in pediatric patients wiht relapsed or refractory acute lymphoblastic leukemia. *Haematologica*. 2017;102(s2):abstract S477.

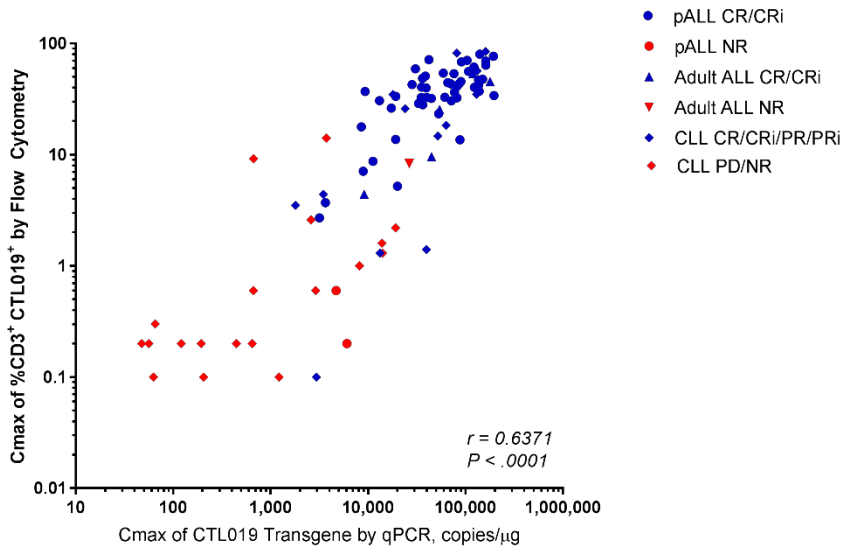
2. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med*. 2015;7(303):303ra139.

Supplemental Figure 1. Correlation between qPCR and flow cytometry in PB. (A) AUC0-28d. (B) Cmax.

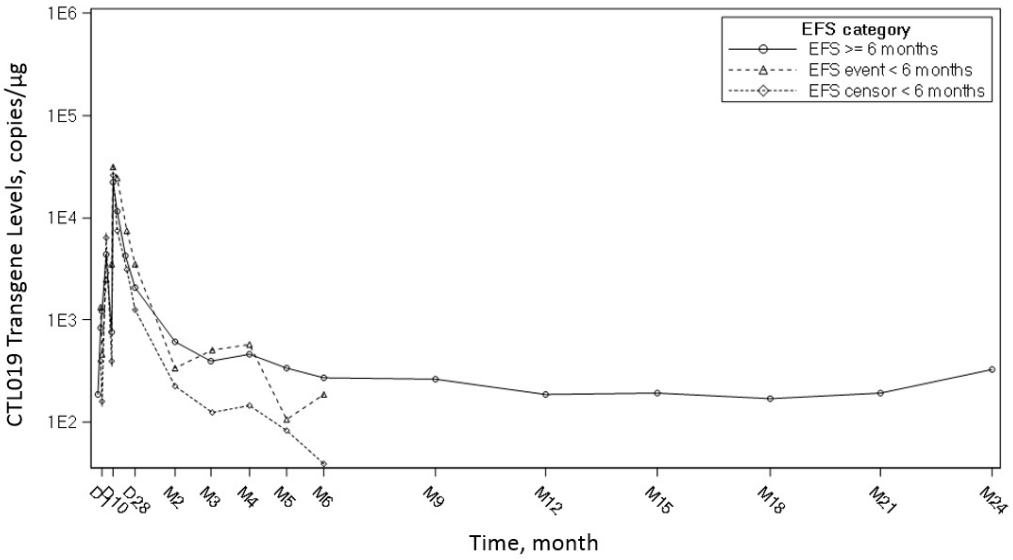
A.



B.

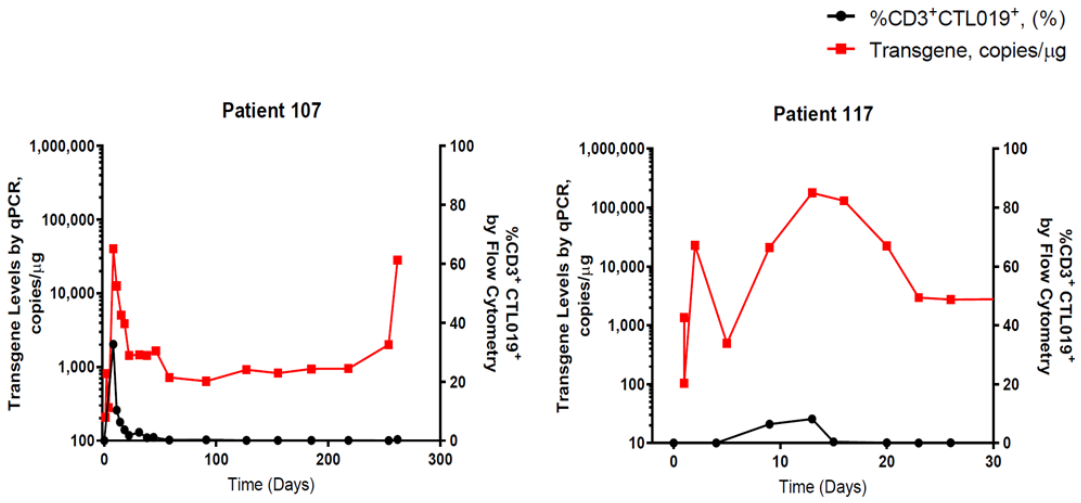


Supplemental Figure 2. Relationship of cellular kinetics, as determined by qPCR, to duration of remission in pediatric B-ALL. Patients who received additional doses of CTL019 after day 28 and those who had not reached the 6-month assessment were censored.

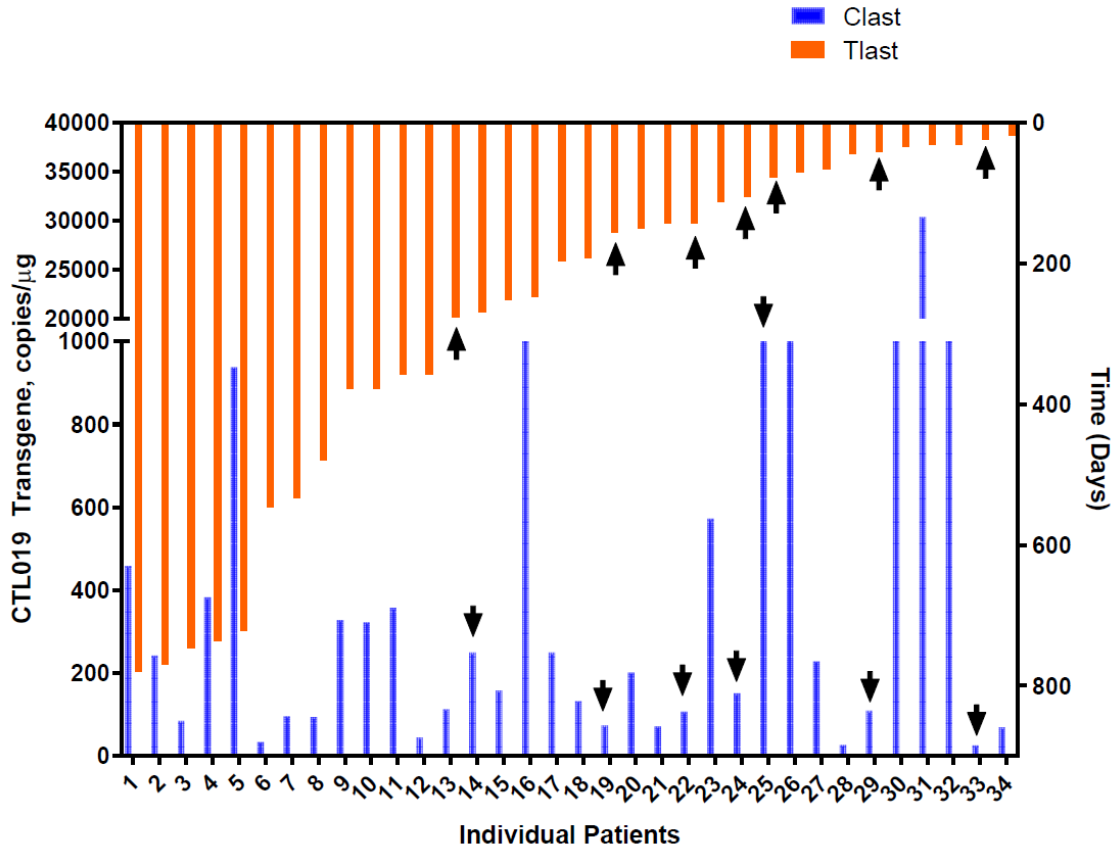


Supplemental Figure 3. Cellular kinetic profiles from individual patients with pediatric B-ALL.

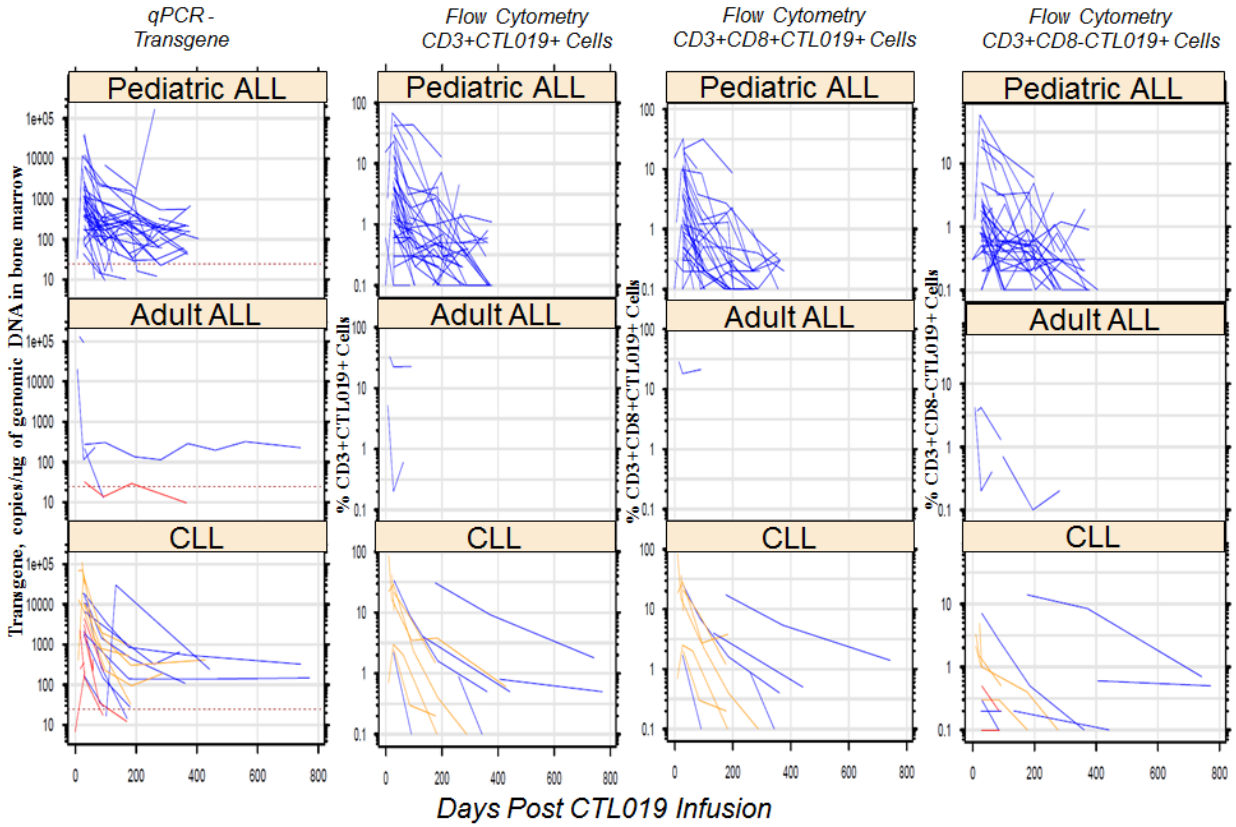
(Left) Measured by qPCR. (Right) Measured by flow cytometry. In a CR patient with pediatric B-ALL with a CD19^{-} relapse at 9 months, a secondary peak was observed at around 9 months in the semilogarithmic scale (patient 107; left panel). It was later determined by flow cytometry that this peak was the result of CAR-expressing leukemic B cells associated with this CD19^{-} ALL relapse.[35] However, by flow cytometry, this patient had a Cmax comparable to the geometric mean for the NR population and an AUC0-28d approximately 4.7-fold lower than the geometric mean for the NR population (eg, patient 117; right panel).



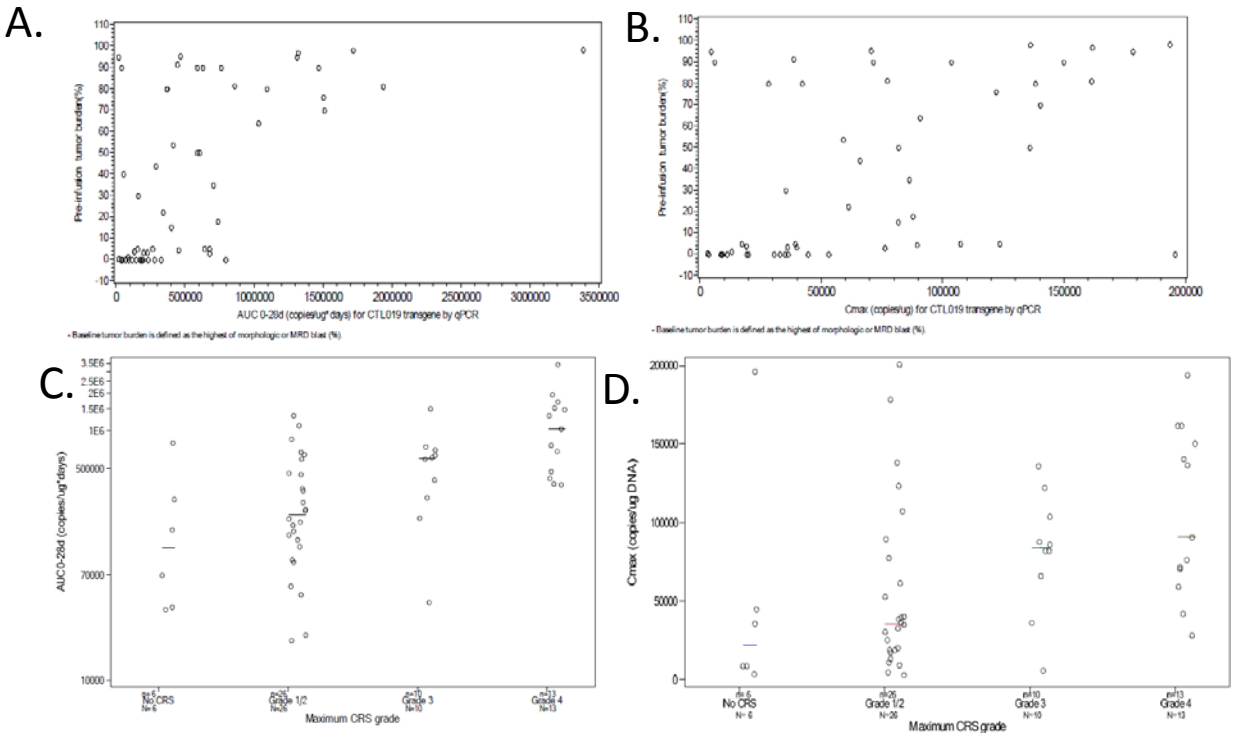
Supplemental Figure 4. Persistence of CTL019 as determined by Clast and Tlast in individual patients with pediatric B-ALL. Arrows represent the patients that have $CD19^{-}$ relapses. All other patients in this figure have maintained CRs.



Supplemental Figure 5. Cellular kinetic in BM for patients with pediatric B-ALL, adult ALL, and CLL. (A) CTL019 transgene, (B) %CD3$^+$ CTL019$^+$, (C) %CD3$^+$ CD8$^+$ CTL019$^+$, and (D) %CD3$^+$ CD8$^-$ CTL019$^+$. CTL019 transgene levels were measured by qPCR and %CD3$^+$ CTL019$^+$ and its CD8$^+$ and CD8$^-$ subsets were measured by flow cytometry.



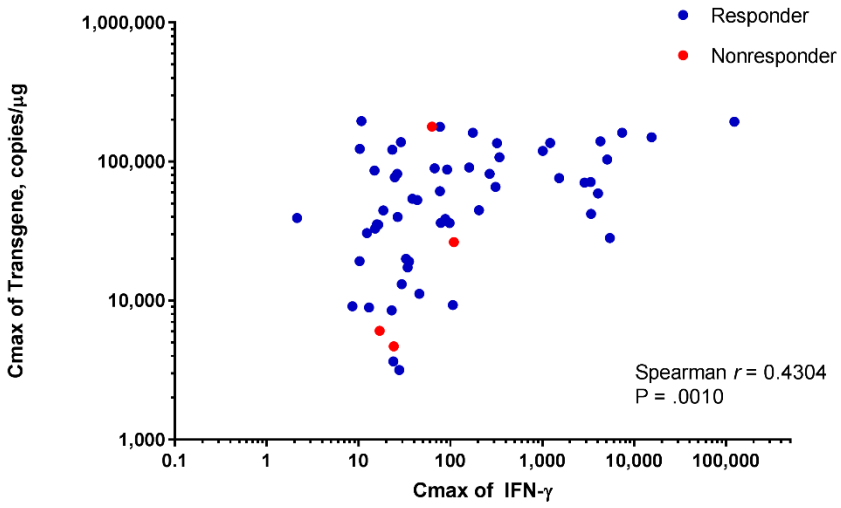
Supplemental Figure 6. Relationship between preinfusion tumor burden and CRS grades with CTL019 exposure as determined by qPCR for patients with pediatric B-ALL.



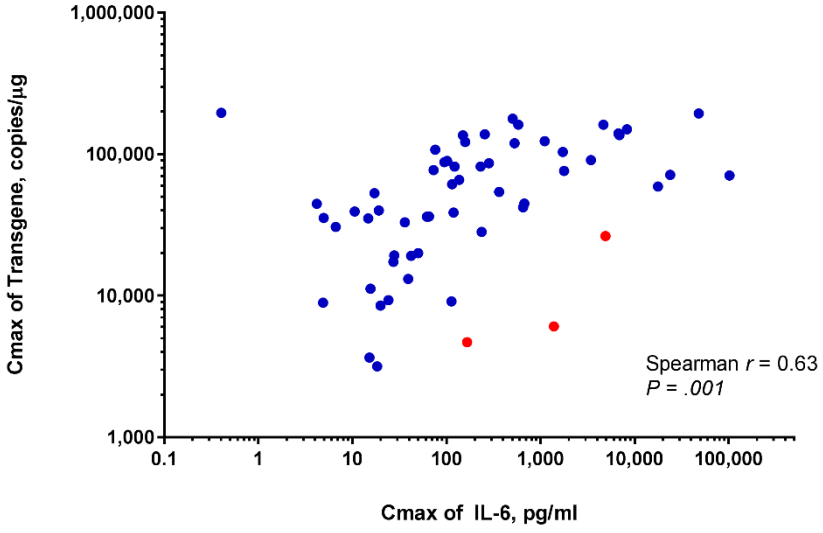
Supplemental Figure 7. Relationship of Cmax to serum cytokine levels in pediatric B-ALL and adult ALL.

(A) Interferon γ (IFN-γ). (B) IL-6.

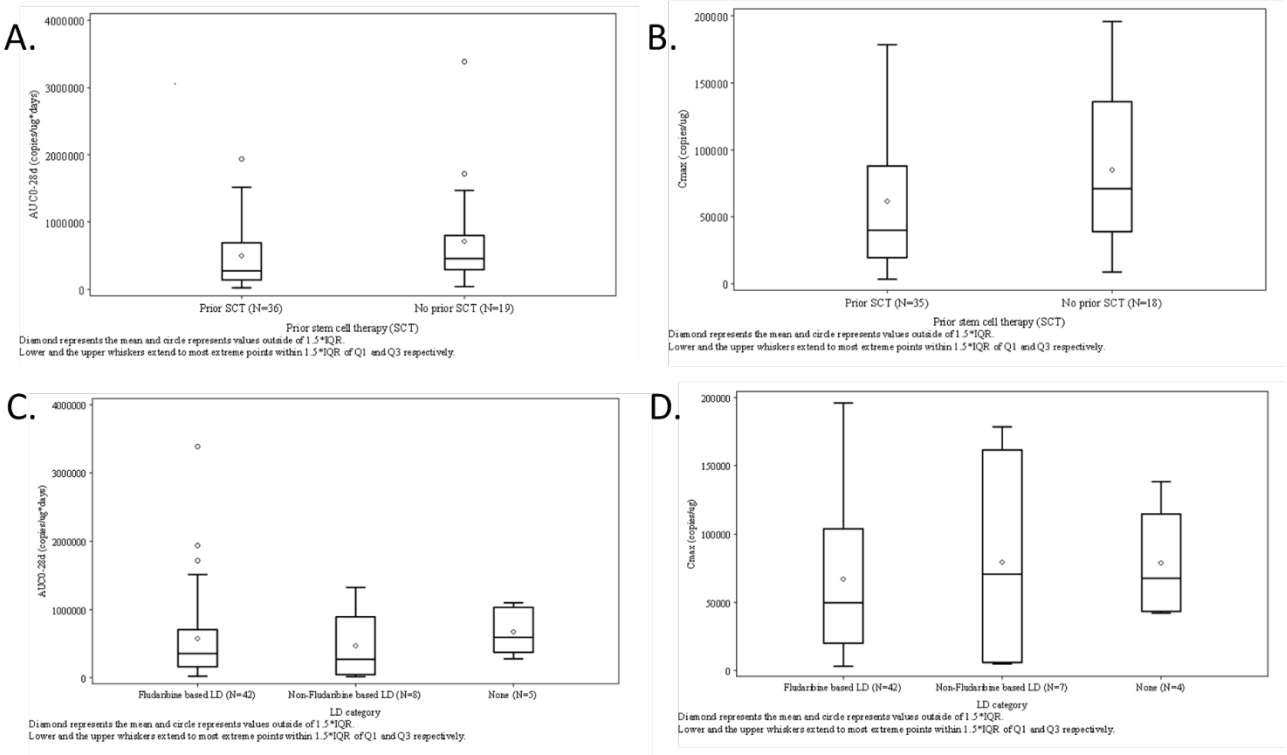
A.



B.

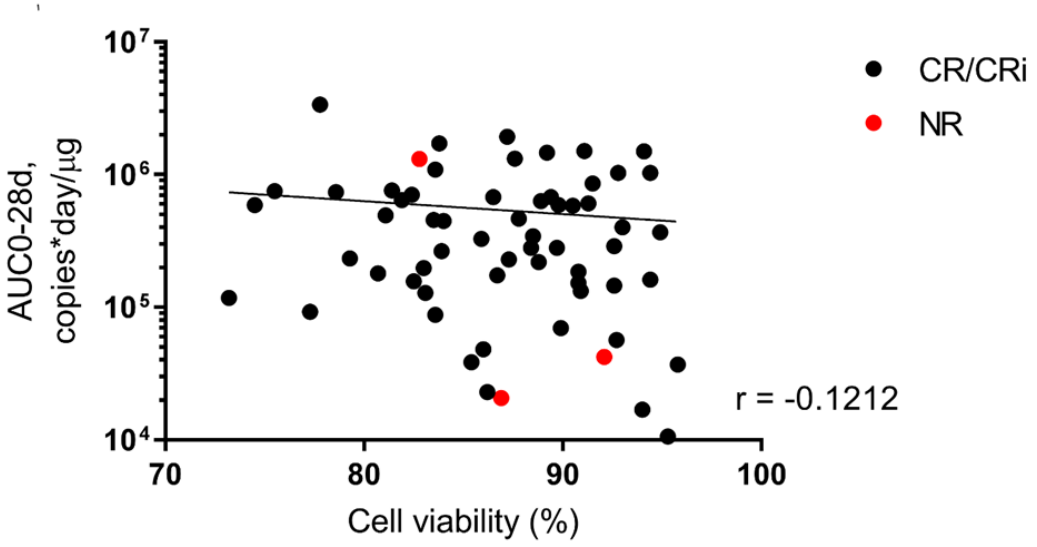


Supplemental Figure 8. Impact of SCT and chemotherapy regimen on cellular PK. Boxplot of (A) AUC0-28d and (B) Cmax for patients with prior SCT (n=36) and patients with no prior SCT (n = 19). Boxplot of (C) AUC0-28d and (D) Cmax for patients treated with fludarabine based LD chemotherapy (n = 42), non-fludarabine based LD chemotherapy (n = 8) or no chemotherapy (n = 5).

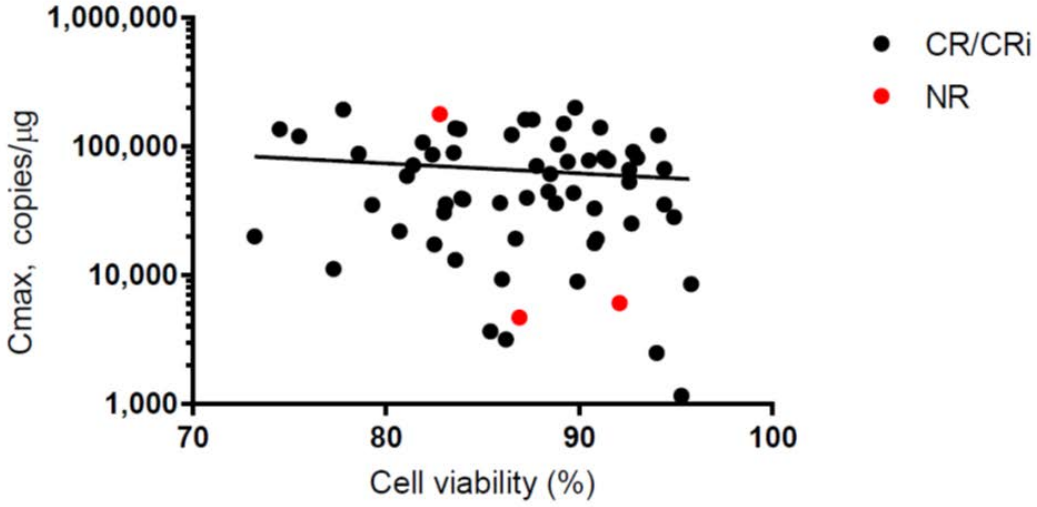


Supplemental Figure 9. T cell viability and expansion (A) AUC0-28d) and (B) Cmax.

A.



B.



Supplemental Table 1. Overview of studies used to investigate CTL019 cellular kinetics

Study	Study Design	Indication (No. of patients for cellular kinetic analysis)	Total Cell Dose	Analysis Method for Transgene Levels/Transduced Cells for Cellular Kinetic Parameter Estimation
NCT01626495[a]	Phase 1/2a	R/R ALL (n = 55)	Up to a total dose of 1.5×10^7 to 5×10^9 (0.3×10^6 to 1.0×10^8/kg) total cells	qPCR, flow cytometry
NCT01747486	Phase 1	R/R CLL (n = 28)	Arm 1 (high dose): target dose of 1×10^8 to 5×10^8 CTL019-transduced cells Arm 2: target dose of 1×10^7 to 5×10^7 CTL019-transduced cells	qPCR, flow cytometry
NCT01029366	Phase 1/2	R/R CLL (n = 14) R/R ALL (n = 6)	Single dose of CTL019 (consisting of approximately 5×10^9 total cells, with a minimal acceptable dose for infusion of 1.5×10^7 CTL019 cells) was to be given to patients as fractions (10%, 30%, and 60% of the total dose) on days 0, 1, and 2	qPCR, flow cytometry

[a] An additional 5 patients (3 patients with CNS3 and 2 patients with lymphoma) treated in NCT01626495 were not included in the analysis.

Supplemental Table 2. Cellular kinetic terms

Term	Description
Cmax	Maximum levels of gene-modified cells achieved in vivo following infusion of CTL019
Tmax	Time at which maximum expansion occurs
AUC0-28d	Total expansion during the first 28 days following infusion of CTL019
Tlast	Time corresponding to the last measurable transgene level (or $CD3^+$ $CTL019^+$ cells)
Clast	Last measurable concentration
T1/2	Apparent half-life, representing the terminal decline of circulating CTL019 cells

Supplemental Table 3. Patient characteristics

Demographic Variable Statistics	Patients With Pediatric B-ALL n = 55	Adult Patients With ALL n = 6	Patients With CLL n = 42
Age, years			
Mean (SD)	11.5 (4.91)	50.2 (15.77)	63.2 (6.81)
Median (range)	11.0 (1-24)	50.5 (25-71)	62.0 (50-77)
Sex, n (%)			
Female	25 (45.5)	1 (16.7)	8 (19.0)
Male	30 (54.5)	5 (83.3)	34 (81.0)
Race, n (%)			
Caucasian	46 (83.6)	5 (83.3)	42 (100)
Black	4 (7.3)	1 (16.7)	0
Other	5 (9.1)	0	0
Previous stem cell transplant, n (%)			
No	19 (34.6)	6 (100.0)	41 (97.6)
Yes	36 (65.4)	0	1 (2.4)
No. of prior regimens[a]			
1	1 (1.8)	0	1 (2.4)
2	5 (9.1)	2 (33.3)	6 (14.3)
3	11 (20.0)	2 (33.3)	12 (28.6)
4	15 (27.3)	0	4 (9.5)
> 4	23 (41.8)	2 (33.3)	19 (45.2)
Median (range)	4 (1-8)	3 (2-5)	4 (1-9)

[a] A single patient with pediatric B-ALL had prior CAR T-cell therapy; however, the transgene from the previous CAR T-cell therapy did not cross-react with the qPCR assay used in this study.

Supplemental Table 4. Summary of cellular kinetic parameters in PB by flow cytometry

	Pediatric ALL		Adult ALL		CLL			Pooled ALL and CLL[a]	
	CR/CRi	**NR/PD**	**CR/CRi**	**NR/PD**	**CR/CRi**	**PR/PRi**	**NR/PD**	**CR/CRi/PR/PRi**	**NR/PD**
AUC0-28d, % × days									
n	50	2	5	1	6	6	9	67	12
Geometric mean (CV%)	260.4 (117.6)	15.5 (496.2)	183.2 (150.2)	55.5	57.5 (1085.7)	221.9 (270.8)	9.4 (218.8)	218.4[b] (177.9)	11.9 (230.7)
AUC0-84d, % × days									
n	29	0	5	1	3	6	5	43	6
Geometric mean (CV%)	460.5 (116.3)	—	242.3 (250.1)	55.5	380.1 (1515.4)	648.9 (225.6)	17.2 (183)	442.4 (163.4)	20.9 (175.3)
Cmax, %									
n	51	2	5	1	6	7	21	69	24
Geometric mean (CV%)	31.8 (90)	0.3 (91)	19.2 (144.9)	8.3	9.2 (262)	10.6 (2210.6)	0.5 (281.9)	24.6[b] (176.3)	0.5 (297.1)
Tmax, days									
n	51	2	5	1	6	7	21	69	24
Median	11	10.5	11	10	14	14	15	11.0	14.0
Range	7-31	8-13	7-15	—	13-20	11-30	1-43	7.0-31.0	1.0-43.0
T1/2, days[c]									
n	49	2	0	0	0	0	0	49	2
Median	10.4	20.2	—	—	—	—	—	10.4	20.2
Range	0.8-99.1	7.4-33	—	—	—	—	—	0.8-99.1	7.4-33.0
Clast, %									
n	38	1	5	1	6	7	20	56	22
Geometric mean (CV%)	0.4 (290.2)	0.1	0.5 (1082)	8.3	0.2 (69.4)	0.3 (136.9)	0.2 (140.9)	0.3 (261.6)	0.2 (209.2)
Tlast, days									
n	38	1	5	1	6	7	20	56	22
Median	120	29	65	10	161	176	25	120.0	25.0
Range	30-780	—	29-651	—	26-361	14-429	8-86	14.0-780.0	8.0-86.0

[a] Data were pooled because there were insufficient data for statistical testing for each population separately. Although there are disease-specific differences between ALL and CLL, the trend for higher exposure among patients with CR/CRi and PR/PRi vs those with NR/PD across both diseases was the rationale for pooling the data.

[b] $P < .0001$ (CR/CRi/PR/PRi vs NR/PD).

T1/2 by flow cytometry of %$CD3^+$/$CTL019^+$, %$CD3^+$/$CD8^+$/$CTL019^+$, and %$CD3^+$/$CD8^-$/$CTL019^+$ was not determined and may be attributed to loss of detectable CTL019 cells with functional persistence.

Supplemental Table 5. Cellular PK by age.

	< 6 years	≥6 to < 13 years	≥ 13 to < 18 years	≥ 18 years	All patients
AUC0-28d, copies/ug x days					
n	8	28	14	5	55
Geometric mean	231,000	327,000	336,000	434,000	321,000
CV%	203.2	207.3	164.3	108.3	179.8
Cmax, copies/ug					
n	8	26	14	5	53
Geometric mean	35,100	45,900	47,600	55,000	45,300
CV%	133.0	181.6	140.6	87.9	149.0
Tmax, median days					
n	8	26	14	5	53
Median	11.0	11.0	9.0	11.0	11.0
(Min, max)	(7.0, 31.0)	(2.0, 18.0)	(7.0, 13.0)	(8.0, 15.0)	(2.0, 31.0)

Supplemental Table 6. Cellular kinetics by number of doses.

	1 Dose	2 Doses	3 Doses	All patients
AUC0-28d, copies/ug x days				
n	10	16	29	55
Geometric mean	277,000	369,000	313,000	321,000
CV%	230.6	179.4	174.8	179.8
Cmax, copies/ug				
n	9	15	29	53
Geometric mean	45,500	46,600	44,500	45,300
CV%	155.9	176.3	142.9	149.0
Tmax, median days				
n	9	15	29	53
Median	11.0	11.0	10.0	11.0
(Min, max)	(7.0, 16.0)	(2.0, 31.0)	(2.0, 18.0)	(2.0, 31.0)